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# THE OXYGEN MINIMUM AND THE GERMINATION OF XANTHIUM SEEDS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 152

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(WITH ONE FIGURE)

Although delayed germination has received considerable attention during the last few years from investigators both in America and Europe, not much has been accomplished toward solving the problems presented by this phenomenon. This may be due in large measure to lack of exact, or at least quantitative, methods of investigation, and to mental attitude which may modify the interpretation of results. Vitalistic interpretations of phenomena may at times prevent a close analysis of the physical and chemical phenomena which condition the manifestations of life, thus preventing the solution of the real problems.

During the last two years the writer has been engaged in an investigation of the relation of oxygen pressure to the germination of *Xanthium* seeds. The need of oxygen for germination and growth of organs in the higher plants has been under discussion for some time. TAKAHASHI (27) has shown that rice can germinate in complete absence of free oxygen, and CROCKER (5) has shown the same to be true for the seeds of certain water plants, as *Eichhornia* and *Alisma Plantago-aquatica*. NABOKICH (20) has experimented on the hypocotyls of *Helianthus annuus*, *Vicia Faba*, and *Phaseolus vulgaris*, and concludes that the organs of higher plants generally are able to grow in entire absence of oxygen. A rather small number of species was used from which to draw sweeping conclusions. LEHMANN (18) investigated the anaerobic growth of the organs of higher plants, and found that there is practically no growth in epicotyls at 1 or 2 mm. of atmospheric pressure. In some instances, as with *Helianthus annuus*, growth of the hypocotyl occurred in total absence of oxygen in distilled water at a temperature above 25°. In 0.5-1 per cent sugar solution, growth occurred even at 20°, but was slight. A number of plants, *Vicia Faba*,

*Pisum sativum*, *Brassica Napus*, *Lupinus albus*, and *Cucurbita*, failed to grow in total absence of oxygen, either in water or in sugar solution, at any temperature. It is evident that the organs of higher plants vary according to species in their need of oxygen for growth and germination.

The results obtained with *Xanthium* seeds emphasize this variability, as will be shown later. A preliminary report of this work (26) was published some time ago, since which time the work there outlined has been largely completed. The problem was suggested by Dr. WILLIAM CROCKER, and has been pursued under his direction at the Hull Botanical Laboratory. It is a pleasure to acknowledge my indebtedness to him for many helpful suggestions during the course of the experiments, and for making during my absence from the University of Chicago some accurate determinations of the vapor pressure present in the apparatus under experimental conditions.

### Historical

The literature on delayed germination has been reviewed so recently by others that a detailed account of the earlier work is not necessary here. Some of the earliest experiments were carried on by NOBBE (21) during the decade 1870-1880, in connection with the testing of agricultural seeds. A little later NOBBE and HÄNLEIN (22) tested many weed seeds, obtaining remarkable results; and finally HÄNLEIN (11) reported on a large number of seeds which he kept in germinative conditions for 1173 days, many of which showed a fraction of 1 per cent of germination, while of *Phyteuma spicatum* L. and *Primula elatior* Jacq. not a single seed germinated in that time. These investigators recognized that the testa in some cases excluded water and prevented germination. But when the testa allowed water to enter, which was not infrequent, and still no germination occurred, both writers refer to this phenomenon as an inexplicable "Rätsel."

HÄNLEIN recognized another category of behavior, however, in which the resistance of the seed to germinative conditions is not external and mechanical, but is internal and protoplasmic. The character of the ovule, the origin, character, and age of the fertiliz-

ing pollen, the nutrition of the parent plant, accidents of sun and shade, moisture and dryness of soil, high and low altitude, weather conditions during ripening, the time of harvesting, and subsequent handling of seeds are suggested as influential factors in determining germination behavior.

The germination of *Xanthium* seeds was first investigated by ARTHUR (1), who noted the dimorphic character of the two seeds in the bur, and found that the lower seed germinated in the spring after ripening, while the upper seed germinated the second year or later. Believing that the testa was too nearly alike in both seeds to cause this difference, and finding much more reducing sugar in the lower seed than in the upper after exposure to germinative conditions, he suggested that enzymes were produced in the lower seeds more readily than in the upper, and that the delayed nutrition of the embryo of the upper seed was the probable cause of the delay in germination.

MASTERMAN (19) tested the germination of *Xanthium* in some field experiments during several years, and showed that over 90 per cent of the burs grew both seeds the same year, thus contradicting the statement of ARTHUR that the upper seed was usually delayed till the second year or later. MASTERMAN'S experiments were not critical, but the results that he and ARTHUR obtained are readily explained and harmonized in the light of more recent investigation.

PAMMEL and LUMMIS (24) found that weed seeds germinated a much higher percentage of seeds after having been frozen than before. In the case of *X. canadense*, they found that none of the fresh seeds germinated during the fall, but after freezing and thawing during the winter, over 50 per cent of them germinated. FAWCETT (7) obtained similar results with many kinds of weed seeds, the percentage of germination being increased, and the "period of dormancy" shortened by freezing again and again.

If the upper cocklebur seed is delayed till the second spring or later, it is because it has resisted the forces of disintegration which finally make possible the entry of oxygen in sufficient quantity for germination, or has not experienced the high temperatures which CROCKER has shown will cause the germination of uppers with

coats intact. Such resistance may be the usual thing in seeds collected and kept at an even temperature during the first winter. But in nature the extremes of winter climate must often destroy the integrity of the coats of these seeds, thus admitting the oxygen necessary for growth. And even if the seed coat is intact, the high temperature often experienced during the spring and early summer is sufficient to germinate the upper seeds if they are near the surface of the ground, since temperatures between 30 and 35° C. cause germination of the uppers with coats intact. Thus it is seen that the results of ARTHUR and MASTERMAN may both be correct under proper circumstances, and the discrepancy in their results is readily accounted for.

Later CROCKER (4), in testing ARTHUR's enzyme theory, discovered that the cause of delay in the upper seed lay in the seed coat, which, though not excluding water, restricted the supply of oxygen to the embryo to such an extent that growth was temporarily suppressed. He tested many other seeds which showed delayed germination and found that, contrary to the usual opinion, in the majority of cases the coat was responsible for the delay by exclusion or restriction of oxygen or water. He pointed out that the restriction of oxygen for the embryo by the testa gave these seeds an exceptionally high minimum temperature for germination, and that, since the seed coats of uppers and lowers differed in the degree of restriction, two minimum temperatures exist for each seed, one with the coat intact, the other with the coat removed. He suggested that high temperatures caused germination by hastening the diffusion of oxygen through the seed coats; but since it is shown in this paper that a rise of temperature decreases the amount of O<sub>2</sub> demanded for growth, the question as to the influence of temperature on diffusion in this particular case is an open one.

High oxygen pressures brought about germination in a short time, but not in the usual manner. The cotyledons elongate sooner than the radicle, due to the thinness of the testa at the distal end of the seed permitting the diffusion of oxygen more readily over the cotyledons.

CROCKER (5) tested seeds of water plants also, and showed that in many instances the delay in germination was due to coat char-

acters, which exclude or restrict the supply of water, rather than to embryonic characters.

At about the same time certain German investigators published a number of papers dealing with similar problems, which are of interest chiefly because of the interpretations of their results. CORRENS (3) found a higher percentage of germination in the disk seeds of *Dimorphotheca pluvialis* than in the ray seeds. He ascribed the difference in percentage of germination to the different constitution of the embryos, but CROCKER showed that in the dimorphic seeds of *Axyris amaranthoides* the non-winged seeds were delayed by coat characters, and that the percentage of germination did not differ when the coats were broken, both showing 100 per cent in three days.

ERNST (6), working with seeds of *Synedrella nodiflora*, showed that light of various intensities and refrangibility affected the length of time necessary for germination, and he attributed not only percentage of germination but also the length of time necessary for germination to the constitution of the embryo.

FISCHER'S (8) paper on the influence of hydrogen and hydroxyl ions on seeds of aquatic plants appeared shortly before CROCKER'S work on the seeds of the same plants. FISCHER interpreted his results as showing that the ions stimulated and awakened the supposedly dormant protoplasm to activity, and thus caused germination to occur. But as already intimated, it was shown by CROCKER that the protoplasm of the seeds of these aquatic plants is not dormant, and needs no stimulus except the necessary conditions for germination, which are supplied if the testa is removed or broken.

OSTENFELD (23) found that digestive enzymes of birds favored the germination of seeds, but he refrained from ascribing the results to the effect of the enzyme on the embryo, saying that the question raised by the widely different interpretations of FISCHER and CROCKER was an open one.

Following the work of ERNST mentioned above, KINZEL (12-16) has shown that light is a factor in the delayed germination of many seeds. The data accumulated show that in some way light of various intensities and refrangibility modifies the seed with the testa intact. The interpretation in all cases ascribes the results

to the protoplasmic characters of the embryo, which are supposed to be changed so that the seed becomes more active or less active by exposure to light. The uses of such terms as "lichtmüde" and "dunkelhart" as applied to protoplasm gives one the characteristic viewpoint.

Very recently GASSNER (9, 10) has studied the effect of light on the germination of some South American Gramineae. His interpretation is in harmony with that of KINZEL, the effect of the light being considered as exerted upon the embryo.

In none of these recent investigations have the methods been sufficiently refined to locate with certainty the cause of the delay. Before the real truth in regard to the cause of delay in many of these instances can be ascertained, more exact and analytical methods of procedure must be brought to bear upon the problem. A careful reinvestigation of some of these cases will not only probably locate the causes, but also reveal the nature of the causes of delay.

### Materials and methods

This investigation of the germination of the seeds of *X. pennsylvanicum* and *X. glabratum* was undertaken after the discovery had been made that the oxygen pressure necessary for germination with testa removed was lower than the results CROCKER obtained with them intact would seem to indicate, and with the knowledge that the seeds of some higher plants, as *Alisma*, *Eichhornia*, rice, etc., could germinate in entire absence of free O<sub>2</sub>. Seeds were collected in various places. Those used during the first season were secured in vacant lots in and near Chicago during the spring of 1909. One lot of seeds had been collected in the autumn as soon as ripe, and were kept in cool dry storage during the succeeding winter and spring. During the second season, seeds were used which had been collected in Lexington, Ky., in November 1909, and kept in an unheated dry room until June 1910. These seeds would all be included in Gray's *X. canadense* Mill., but BRITTON's treatment of the genus is certainly more satisfactory than GRAY's, and I use the names *X. pennsylvanicum* Wallr. and *X. glabratum* Britton as in the BRITTON *Manual*. I am indebted to Dr. J. M. GREENMAN for examining the seeds and

permitting me to see and compare my materials with the genus collection of *Xanthium* in the Field Museum of Natural History.

In determining the minimum oxygen pressure required for the initiation of protoplasmic activity, reduction of total atmosphere was employed. The apparatus used at first was a slightly modified form of that used by SCHAIBLE (25) in his experiments on the germination and growth of various plants at reduced atmospheric pressures. In order to control the light, the germinators were at

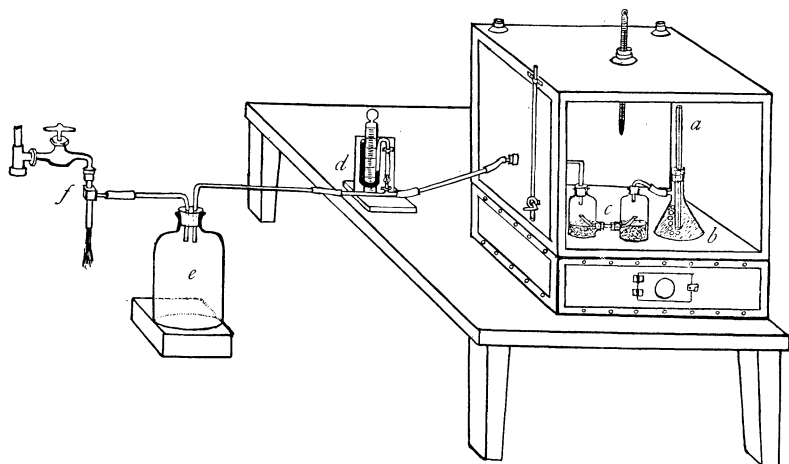


FIG. 1.—Diagram of apparatus: *a*, capillary tube; *b*, wash bottle; *c*, two germinators with wet cotton, for lowers and uppers; *d*, pressure gauge; *e*, vacuum chamber to secure immediate low pressure when the germinators are started; *f*, aspirator run by constant level system with a fall of 45 feet.

first surrounded by opaque black paper; but later, when temperature was found to be a very important factor in the result, and while testing the influence of temperature on the oxygen minimum, light and temperature were both controlled by placing the germinators in a water bath provided with a sensitive electric thermostat. A diagram of the apparatus is shown in fig. 1.

The seeds were germinated on moist absorbent cotton, and a constant current of air was drawn into the apparatus through long pieces of capillary tubing by means of powerful aspirators. In order to overcome the drying effects of a low atmosphere, a dish of water was set in each germinator in addition to the saturated



absorbent cotton on which the seeds lay. This method was discarded for the temperature and pressure experiments, as a more satisfactory arrangement consisted in immersing the lower end of the capillary tube in a flask of water, thus drawing the atmosphere through water before it entered the germinator. This method had the added advantage of showing at once whether the flow of air through the capillary tubes was being interfered with by dust particles. The air was kept moist in this way, but owing to the very rapid exchange of gas in the apparatus, the atmosphere was not saturated. The water which ran the aspirators had a fall of 45 feet, and was furnished through a separate constant level system which gave the aspirators uniform power.

The seeds were in all cases prepared for experimentation by soaking them in ice water, far below the minimum temperature for germination, for at least 12 hours, after which the testa was removed carefully, without injury to the seeds. They were exposed to constant conditions for 10 days, and the elongation of the hypocotyl and the geotropic response was used as the criterion of germination.

Temperature series at various pressures were run at  $31^{\circ}\text{C.}$ , and the influence of fluctuating temperature was determined by using a fluctuation of  $25\text{--}40^{\circ}\text{C.}$

At the low pressures and high temperatures employed, the evaporation of water in the apparatus is very rapid. In determining the actual oxygen pressures to which the seeds are subjected, it is necessary to know what volume of air is drawn through the apparatus in a given time, and what part of the gas pressure is due to water vapor. The atmosphere is not saturated, for if it were saturated at these reduced pressures, the water vapor pressure alone would be much higher than the total pressure used. The amount of air at normal pressure entering the apparatus is 3–5 liters per hour, a very rapid exchange, since it means 8–12 times that volume per hour within the germinators due to expansion under diminished pressure. Under such circumstances it is not surprising to find that the water vapor is removed so rapidly that the saturation point is not approached. It was clearly demonstrated, however, that water was not a limiting factor in these experiments.

Any increase of the aqueous vapor about the seeds was invariably attended by a marked decrease in the percentage of germination and amount of growth, due to the fact that such increase necessarily reduces the oxygen supply of the germinating seeds. In spite of the rapid evaporation and removal of the water, there is sufficient moisture present to bring about all the growth changes which the oxygen supply will permit, as was shown by repeated tests.

In correcting the pressures for water vapor, the only practicable method is to measure directly the amount of dry air passing into the apparatus per hour, and the amount of water vapor drawn from it in the same length of time, then calculate the proportion of each gas in the gram molecular volume. A concrete example will make the method clear. At 88 mm. pressure, 30° C., the dry air drawn through the apparatus measured at 20° C., barometer 745 mm., is 4.37 liters per hour. Reducing this volume to standard temperature and pressure gives 3.9 liters. This volume is 0.178 of the molar volume. The amount of water vapor present with that amount of air drawn from the apparatus as determined by phosphorus pentoxide absorption was 1.65 grams per hour; and this amount is readily found to be 0.086 of the molar volume. The dry air and water vapor together amount to 0.264 of a mole per hour. The pressure recorded by the manometer is 88 mm. Of this amount,  $178/264$  (59.3 mm.) is air pressure,  $86/264$  (28.7 mm.) aqueous pressure. The oxygen pressure is then readily obtained, as oxygen constitutes 20.93 per cent of the atmosphere. The corrections were made on the basis of the average of three determinations, and are therefore fairly reliable.

To determine whether reduction of pressure *per se* has any effect on germination, hydrogen gas with a low oxygen content was admitted to the chambers containing the seeds. The hydrogen was imported by the Linde Air Products Co., of Buffalo, N.Y., and was found to contain 2.34 to 4.7 per cent of oxygen. The gas with more than 2.5 per cent of oxygen was of little use, because the oxygen pressure was so high that no comparison with the reduced pressure experiments could be made. This hydrogen was under 120 atmospheres of pressure, but was controlled by high

pressure valves so that a small stream of gas at normal pressure ran constantly through the germinators.

The gas was washed by passing it successively through potash bulbs containing concentrated potassium permanganate solution, and 33 per cent potassium hydroxide. The whole series of coils and jars was packed in ice for 12 hours after the hydrogen began to flow, and the gas was carefully analyzed by phosphorus absorption until it was found to be coming from the apparatus with as low oxygen content as when taken directly from the tank, at which time the temperature was allowed to rise sufficiently for germination. In this way the possibility of the initiation of germination in only a partially replaced atmosphere was precluded. The apparatus was quickly brought to ordinary temperature, and kept constant at 21.5° C. by allowing a current of water from Lake Michigan to flow over and around the potash coils and germinators. Reduced atmosphere experiments at the same temperature were run at the same time, but the correction for water vapor in these series makes a direct comparison with the results in hydrogen impossible. However, comparison of these hydrogen results with other low pressure series run in the same way, makes it possible to draw trustworthy conclusions.

Attempts were made to test the after-ripening of *Xanthium* seeds of different ages, from green to a year old, at normal and reduced pressures. These experiments were not extensive, and on the whole not very satisfactory; but the results indicate that only very slight changes occur.

In all cases control cultures were employed. In measuring the growth of the controls, a difficulty presented itself. At full atmospheric pressure the roots of *Xanthium* seedlings penetrate the substratum of cotton with innumerable branches, impossible of disentanglement and accurate length measurement. In all these cases only the unbranched portion of the plant was measured. In all the experimental plants the total growth in length was easily measured and is so recorded.

### Experiments

As indicated above, the experiments proceeded along four lines: to determine the minimum oxygen pressure necessary for germina-

tion, and to determine the influence of temperature, pressure, and after-ripening on that minimum. Since the first of these determinations is that on which the others bear, that portion of the work will be considered immediately.

#### THE MINIMUM OXYGEN PRESSURE

The tabulated results of these experiments are given in the preliminary report, but for convenience they are presented here again, with one addition, a test with *X. glabratum* (all others are *X. pennsylvanicum*) at 85 mm., and with correction of the oxygen pressure for aqueous vapor.

TABLE I  
DURATION OF EXPERIMENTS 10 DAYS

MANOMETER PRESSURE (MM.)	AQUEOUS PRESSURE (MM.)	ATMOSPHERIC PRESSURE (MM.)	OXYGEN PRESSURE (MM.)	TEMPERATURE (C.)	PERCENTAGE GERMINATION				GROWTH IN LENGTH OF HYPOCOTYL (MM.)			
					Lowers	Controls	Uppers	Controls	Lowers	Controls	Uppers	Controls
99.....	16.4	82.6	17.3	19-22	75.0	100	45	95	14.5	30.0	4.9	23.3
90.....	16.3	73.7	15.4	21-22.6	80.0	95	50	100	22.8	45.9	4.3	37.8
85.....	16.25	68.75	14.39	21.5	56.6	93.3	23.3	100	42.8	103.64	10.5	94.9
72*.....	16.14	55.86	11.69	20-28	45.0	100	20	100	11.5	46.0	9.4	33.6
72.....	16.14	55.86	11.69	20-22	30.0	95	0	100	6.36	28.5	0.0	22.0
28*.....	15.27	12.73	2.66	21.5-24.5	0.0	100	0	95	0.0	37.8	0.0	28.8

\* Temperature not controlled.

The table shows at once that there is a marked difference between the upper and lower seeds in percentage of germination and amount of growth under identical conditions, this difference being the expression of a decided difference in the oxygen need of the two seeds for germination. The difference in percentage of germination at each pressure and temperature used is fairly constant, the lowers germinating about 30 per cent more seeds than the uppers in each experiment. The difference in the oxygen need is several millimeters, the lowers requiring less oxygen than the uppers for the initiation of activity. The oxygen minimum for the uppers is approximately 12 mm., at 21° C., while the minimum for the lowers is about 9.5 mm.

It is important to notice the relation which this physiological difference in the embryos of the two seeds bears to the difference

in their seed coats. CROCKER found that restriction of the oxygen supply by the testa was the main cause of delay in germination, and that difference in the degree of exclusion by the testa of upper and lower seed accounted for the difference in delay of the two seeds. But this embryonic difference, which is clearly demonstrated in the table, acts in conjunction with the coat differences in securing a longer delay in the upper than in the lower seeds.

In comparison with the seeds and organs of other plants, the oxygen demand of *Xanthium* seeds is very high. As already noted, most seeds will germinate with not more than a few millimeters of atmosphere, and some germinate without free O<sub>2</sub>, but *Xanthium* requires 9-12 mm. of oxygen, the equivalent of 44-60 mm. of atmosphere. This high demand for oxygen aids in securing delay, for if only a small fraction of a millimeter were needed for germination, the testas might not restrict the supply sufficiently to cause delay. And the difference in demand of the two seeds would secure a longer delay for the uppers than for the lowers, even if the testas did not differ in their power to exclude oxygen from the embryos as they do.

#### TEMPERATURE AND THE OXYGEN MINIMUM

It was found that temperature is a powerful factor in determining the oxygen minimum, slight changes producing marked effects upon the results. The potent influence of temperature in this regard is shown clearly by the two lots of seeds of *X. pennsylvanicum* kept at a pressure of 72 mm. as recorded in table I. The variation noted in these two lots was due to one lot being subjected to a temperature 6° higher than the other during a part of the last two days of the experiment. Previous to that time, the behavior of the two lots of seeds had been almost identical; but 45 per cent of the lowers germinated in the lot which reached 28°, as compared with 30 per cent, the lot which did not go above 22°; and 20 per cent of the uppers germinated as compared with complete failure to germinate at the lower temperature. The amount of growth in each lot shows a similar relationship.

In another instance shown in table I, two lots of seeds were subjected to atmospheric pressures of 90 and 99 mm. respectively,

but the latter lot was subjected to a temperature  $2^{\circ}$  lower on the average than the former. The results show, instead of an increase in percentage of germination and growth in length, as would have been expected from the increased supply of oxygen, a decrease of 5 per cent in the germination of both lowers and uppers, and a considerable decrease in the average growth of the lowers in length.

A number of experiments were performed with the purpose of determining how much effect temperature has on the location of the minimum. The results are presented here in tabular form. For the sake of comparison, one experiment at 72 mm. and room temperature is included at the bottom of the table, which may be compared with the results at 76 mm. and  $31^{\circ}$ , and with those at 75 mm. and  $25-40^{\circ}$ .

TABLE II  
DURATION OF EXPERIMENTS 10 DAYS

MANOMETER PRESSURE (MM.)	VAPOR PRESSURE (MM.)	ATMOSPHERIC PRESSURE (MM.)	OXYGEN PRESSURE (MM.)	TEMPERATURE (C.)	PERCENTAGE OF GERMINATION				GROWTH IN LENGTH OF HYCOTYL (MM.)			
					Lowers	Controls	Uppers	Controls	Lowers	Controls	Uppers	Controls
76.....	29.0	47.0	9.8	31	93.3	100	40.0	100	50.4	102.0	32.75	109.0
65.....	29.1	35.9	7.5	31	60.0	100	43.3	100	33.1	102.0	20.15	109.0
65.....	29.1	35.9	7.5	31	80.0	100	10.0	100	32.4	79.6	24.00	86.6
55.....	28.7	26.3	5.5	31	66.6	100	0.0	100	11.6	79.6	0.00	86.6
75.....	Varies with	temp.	25-40	25-40	93.3	90	56.6	100	37.25	67.4	28.00	70.6
65.....	Varies with	temp.	25-40	25-40	86.6	90	26.6	100	32.9	67.4	17.25	70.6
72.....	16.4	55.86	11.69	20-22	30	95	0.0	100	6.36	28.5	0.0	22.0

The difference between uppers and lowers in percentage of germination is even more pronounced at  $31^{\circ}$  than at  $21^{\circ}$ , amounting in some instances to 60 or 70 per cent more germinations among the lowers than among the uppers. It appears also from table II that the oxygen minimum is considerably lower at  $31^{\circ}$  than at  $21^{\circ}$ , especially for the lower seeds. From the data here presented the oxygen minima have been approximated by mathematical methods. Since a pressure of 65 mm. germinated 10 per cent of the uppers, the oxygen minimum as calculated from the results would be about 6.75 mm. The minimum for the lowers was not accurately determined, but at 55 mm. atmospheric pressure, representing an  $O_2$

pressure of 5.5 mm., there was still 66.6 per cent of germination. The closest estimation possible from the data at hand would indicate an oxygen minimum of about 2.5–3.5 mm. for the lowers at 31°.

The lowering of the minimum is somewhat greater in the lower than in the upper seeds. The oxygen minimum of the uppers is decreased from about 12 mm. to less than 7 mm., while for the lowers the minimum is decreased from 9.5 to about 3 mm. All of these results show that with the increase of temperature there is a decrease in the demand for free oxygen. The probable reason for this will be discussed later. The physiological difference of the embryos of the upper and lower seeds is shown clearly by these experiments, the embryo characters being just as strikingly different as the coat characters; and both sets of characters act together in securing the difference in delay of the two seeds.

Temperatures fluctuating between 25 and 40° are apparently no more effective in producing germination than the constant high temperatures employed. Since the pressure in the germinators remains constant while the vapor pressure fluctuates with the temperature, it is evident that the oxygen pressure fluctuates also, and that its fluctuation is inversely as the temperature, rising as the temperature falls, falling as the temperature rises. CROCKER found such fluctuating temperatures more effective in producing germination than constant temperature of 35° in the upper seeds with testas intact. The fluctuation may render the testa more permeable to oxygen, but in view of the effect of temperature on oxygen demand, the inference cannot be made with certainty.

A peculiar result was observed in all the control experiments at high temperatures. At normal room temperature, both the experimental seeds and the controls show less growth in the uppers than in the lowers; but at high temperatures, whether constant or fluctuating, this relation is reversed in the controls. This is noticed on comparison of lower and upper controls in table II. At the same time, both lowers and uppers of the experimental seeds show less growth in a fluctuating temperature of 25–40° than corresponding lowers and uppers at constant high temperature of 31° and equal pressures. The first two experiments in table II, com-

pared with the two fluctuating experiments in the same table, show this to be true. The decreased growth in the fluctuating temperature in this case is possibly due partially to the decreased oxygen pressure in the germinators as the vapor pressure increases with the rise in temperature.

#### PRESSURE AND THE OXYGEN MINIMUM

The method employed in these experiments has been briefly described, and the results are recorded in table III. Parallel experiments at reduced pressure were run along with the hydrogen tests, the germinators being kept in the same running water at a pressure of 85 mm. The oxygen pressure in the hydrogen and reduced pressure experiments would have been the same but for the aqueous pressure in the latter. The correction for water vapor reduces the  $O_2$  pressure from 17.79 mm. to 14.39 mm., a large enough difference to make the results not directly comparable. The first three tests were with *X. pennsylvanicum*, the remainder with *X. glabratum*.

The first of these hydrogen tests is of little value in determining the effect of pressure on the oxygen minimum, for the oxygen content was 35.25 mm., or more than double that used in any of the reduced atmospheres. Only a slight reduction of the growth is brought about, which would indicate that this amount is probably somewhat below the optimum oxygen pressure for *Xanthium*. NABOKICH has shown that the atmosphere contains considerably more than the optimum oxygen supply for growth in higher plants.

On comparing the results of the remaining hydrogen tests with those at reduced pressures, it is seen at once that there is a higher percentage of germination, and a greater average growth in the hydrogen than in the reduced atmosphere. For instance, in hydrogen 96.6 per cent of the lowers, and 43.3 per cent of the uppers of *X. pennsylvanicum* germinated, as compared with 26.6 per cent of the lowers and 23.3 per cent of the uppers in the 85 mm. atmosphere. *X. glabratum* shows a similar behavior, 56.6 per cent of the lowers, and 23.3 per cent of the uppers germinating in the reduced atmosphere. Is this difference due to a difference in oxygen pressure



TABLE III  
DURATION OF EXPERIMENTS 10 DAYS

MANOMETER PRESSURE (MM.)	PERCENT OF OXYGEN IN HYDROGEN	AQUEOUS PRESSURE (MM.)	ATMOS- PHERIC PRESSURE (MM.)	OXYGEN PRESSURE (MM.)	TEMPERA- TURE (C.)	PERCENTAGE OF GERMINATION				AVERAGE GROWTH IN LENGTH OF HYPOCOTYL (MM.)			
						Lower	Controls	Uppers	Controls	Lower	Controls	Uppers	Controls
	4.70	.....	.....	35.25	21.5-22.5	93.3	86.6	93.3	83.3	94.3	101.8	89.25	105.0
	2.34	.....	.....	17.55	21.5	96.6	93.3	43.3	100	81.8	103.64	47.0	94.9
85.....	....	16.25	68.75	14.39	21.5	26.6	93.3	23.3	100	36.1	103.64	33.9	94.9
85.....	....	16.35	68.75	14.39	21.5	56.6	93.3	23.3	100	42.8	103.64	10.5	94.9
	2.34	.....	.....	17.55	22.0	93.3	100	30.0	100	77.8	88.2	43.2	82.4
85.....	....	16.25	68.75	14.39	22.0	53.3	100	13.3	100	34.0	88.2	19.0	82.4
85.....	....	16.25	68.75	14.39	22.0	90.0	100	13.3	100	38.1	88.2	26.0	82.4

alone, or does the difference in pressure as such have a part in producing this effect? Comparison of the results in hydrogen gas with those at 90 and 99 mm. in table I, which approach most nearly the same oxygen pressure, shows that the difference is probably nearly all due to difference in oxygen pressure, rather than to a difference in barometric pressure. It is possible that great reduction in pressure may affect slightly the percentage of germination and the amount of growth. WIELER (28) came to the conclusion that growth is independent of pressure; and SCHAIBLE (25) observed that reduced pressures increase the rate of growth, but that the influence of air pressure on germination is very slight.

From the data of table III, as compared with the results of the other experiments, it appears that the oxygen minimum is practically the same for any given temperature, whether the reduction is accomplished by reducing the atmospheric pressure, or by dilution with inert gases like hydrogen.

#### AFTER-RIPENING OF XANTHIUM

Several attempts have been made to test the after-ripening of *Xanthium* seeds, with the results here briefly recorded. During October and November 1910, I made a test of the germination of *X. glabratum* at normal pressure, average temperature 23° C., at Transylvania University. Seeds in three different stages were taken as follows: green seeds, so young that the testas were still quite white; seeds which had ripened normally on the plants in 1910; seeds collected in the same locality in 1909. The seeds were prepared for germination as in all the other tests, and the integuments removed carefully. The results show that, at normal pressures at least, the protoplasm does not pass from an inactive state to an ultimately more active one, and that there is no after-ripening in that sense. The seeds averaged as follows in growth in length:

	Lowers	Uppers
1910 green seeds.....	46.0 mm.	37.0 mm.
1910 brown seeds.....	36.0 mm.	31.5 mm.
1909 brown seeds.....	34.3 mm.	30.35 mm.

One experiment with fresh seeds at reduced pressure agrees with these results. The seeds were kept at 90 mm. pressure, tempera-

ture  $21.5^{\circ}$ , December 2-11, 1909. In this case 96 per cent of the lowers, and 33 per cent of the uppers germinated; and the lowers averaged 30 mm. in length, the uppers 10 mm. The controls were injured in some way, but all had germinated. A comparison of these figures with those at 90 mm. pressure, temperature  $21-22.6^{\circ}$  (table I), using seeds almost a year old, shows that fresh seeds have as high a percentage of germination and a more rapid growth than the old seeds under reduced pressure.

In another experiment, however, seeds collected green in 1910 were tested along with seeds a year old, and no germination was secured at 90 or 100 mm. pressure, at  $21^{\circ}$ , in either crop of seeds. In the controls growth was fine, and the 1910 seeds showed a better growth than the 1909, just as reported above for seeds germinated under the same conditions.

It is possible that the after-ripening manifests itself in a change, either in the lowered demand of the embryo for oxygen, or in an increased permeability of the coats to oxygen, or in both at once, the change being too slight to affect the results at normal pressure. Some experience with these seeds in laboratory exercises in the Hull Botanical Laboratory lends force to this suggestion. With freshly ripened seeds having the testas intact, the classes fail to get the usual cotyledonary germination in pure oxygen atmosphere, as was reported by CROCKER for the upper seeds of *X. canadense* after 6 days at  $21-23^{\circ}$ ; but in the winter, several months after ripening, these same seeds gave good cotyledonary germination in pure oxygen. This experience indicates either a decrease in the demand for oxygen by the seed, or an increase in the permeability of the seed coats as ripening progresses, and corroborates the evidence furnished by the experiments on after-ripening.

It is clear from these experiments that there is a slow progressive deterioration of the seeds, manifested in the reduced growth of the seeds as they become older, which after a few years probably causes entire loss of power to germinate. This deterioration seems to be a little more rapid in the lowers than in the uppers, but the physiological difference of the two seeds was very evident in the oldest seeds, and no doubt remains so long as they will germinate at all.

### Discussion

The methods employed in much of the recent work on delayed germination of seeds has not been as exact as is desirable, or even necessary, for the solution of the problems involved in this phenomenon. The factors which are influential in the germination behavior have been investigated qualitatively only, without any attempt to measure them accurately and determine their relations. Moreover, the seed has been regarded too often as an embryo only, which may be affected profoundly by chemical and ethereal stimuli, the testa being considered as negligible on account of its thinness or on other insufficient grounds. In KINZEL's latest paper (16) he claims to have met all the serious criticisms made against his work, but in none of his experiments has he eliminated the testa as a factor. Recently LEHMANN (17) has shown that other stimuli than light can be substituted in its place and produce approximately the same effect. For instance, he found that the effect of 1 per cent Knop's solution in the germination of the seeds of *Ranunculus sceleratus* was much the same, inducing practically the same percentage of germination. If such substitutions of stimuli are possible, what can be stated with certainty as to the cause of delay in these cases? Light and the chemicals undoubtedly affect something, but it may as easily be the testa as the embryo that is changed. Even if the effect is actually produced in the embryo in these instances, the phenomena are not explained by saying that the protoplasm has been rendered "lichthart" or "lichtmüde," as if it were a sort of "weariness of the flesh!" If there is a change in the permeability of the embryo, or other physical or chemical alterations in it which leads to activity or cessation of activity, demonstration of such changes would throw some light on the phenomena.

In these *Xanthium* experiments the determinations have been made with as great exactness as possible, the accuracy lying well within the variability of the seeds themselves, and the work has been almost entirely quantitative. These methods demonstrated clearly the physiological difference between the embryos of the upper and lower seeds, although the difference is so slight as not to affect visibly the germination of the uppers at atmospheric pressure when the testa has been removed. This fact shows the value

of the quantitative method. It may reveal differences existing in various parts of the seed, which would otherwise remain undiscovered, and may in this way lead to results of the highest significance in the explanation of delay in germination. Often only quantitative measurements can detect the factors which determine the peculiarities of behavior.

The oxygen pressure needed to initiate germination in *Xanthium* seeds with coats removed is considerably less than would be expected in view of the rapid exchange of gases which CROCKER found in these seeds with testas intact. However, the amount necessary is large in comparison with the oxygen supply needed by seeds of many other angiosperms. The question as to whether higher plants can grow in absence of oxygen has been much discussed recently. Attention was called to the results of TAKAHASHI on rice, of CROCKER on *Alisma* and *Eichhornia*, of NABOKICH and LEHMANN on the organs of the seeds of many higher plants, in the introduction to this paper. NABOKICH believes that the organs of nearly all seeds of higher plants can grow in absence of free  $O_2$ . *Xanthium* seeds would certainly have afforded him a remarkable exception. It is much more probable, from the data now before us, that the seeds of the higher plants vary largely in the amount of oxygen required for germination, some of them, like *Alisma* *Plantago-aquatica*, rice, and other seeds which grow in media containing little free oxygen, requiring no free oxygen whatever; others, like *Xanthium*, requiring a comparatively large amount; with perhaps the great majority of seeds lying somewhere between them in regard to oxygen need. Such seeds as water plantain stand at the one end of the series, and *Xanthium* perhaps at the other extreme, with all possible intergradations. The experiments which have been carried on with *Xanthium* show that some seeds require a comparatively large amount of free oxygen, thus making each species of seed a problem in itself. There is no one behavior for the seeds of all higher plants, as NABOKICH seems to believe.

There are several facts which need to be considered in connection with the apparent inconsistency between the results I have obtained with *Xanthium* seeds as to the need for oxygen when the

testa has been removed, and those obtained by CROCKER with the same kind of seeds with the testa intact. When the seeds with testas on are allowed to germinate normally, the hypocotyl always elongates first and the root is well developed before the cotyledons begin to grow. That is, there is a strong correlation between the growth of these two organs of the seed. But if the well ripened seeds with coats on are germinated in an atmosphere containing a high percentage of oxygen, the cotyledons instead of the hypocotyl initiate the development of the embryo. The strong normal correlation is reversed by temporary suppression of the hypocotyl, due probably to a more rapid diffusion of oxygen through the very thin distal portion of the testa surrounding the cotyledons than through the thick proximal portion which invests the hypocotyl. The cotyledons thus receive the necessary free oxygen for germination sooner than the more sensitive hypocotyl, with the result that the usual course of growth in the seed is completely changed.

This reversal of a very strong correlation probably requires considerably more oxygen than a normal germination. Not only is the usual behavior overcome, but the part which grows instead of the hypocotyl is a much less sensitive part of the embryo, undoubtedly requiring more oxygen to initiate its activity. In animal cells food storage renders the protoplasm inert, and delays cell division to a very marked degree. It is possible that food storage in the cotyledons has a similar effect on their activity, requiring more energy, and therefore more oxygen, for the growth processes.

Another factor probably responsible for a considerable portion of the gaseous exchange during germination with the seed coats intact is the testa itself. BECQUEREL (2) has shown that the integuments of seeds produce  $\text{CO}_2$  in comparatively large amounts, sometimes greatly exceeding the seeds from which they have been taken in  $\text{CO}_2$  production. For instance, he found that one gram of *Ricinus* integuments exposed to light gave off 18 times more  $\text{CO}_2$  than one gram of seeds with testas removed; that one gram of the testas of *Vicia Faba* gave off 10 times as much  $\text{CO}_2$  as the same weight of decorticated seeds; and that one gram of the coats of the pea produced 25 times as much  $\text{CO}_2$  as a gram of the embryos.

In CROCKER's experiments the measurement of the gaseous exchange was made for seeds with testas on, and therefore included whatever coat  $\text{CO}_2$  production occurred. And, since the thickness of the testa over the hypocotyl practically excludes the oxygen from that organ, while the thinner distal portion of the coat admits the oxygen to the cotyledons first, thus initiating the cotyledonary germination mentioned above, the measurement of the gaseous exchange has dealt very largely with that occurring in the cotyledons. In my experiments the removal of the coats eliminates the coat  $\text{CO}_2$  production; and, since the cotyledons never grow under the conditions of the experiment until after the hypocotyl has elongated, the determination of the oxygen minimum is made for the hypocotyl only. There is no inconsistency, I believe, between the results obtained by CROCKER and those obtained in these experiments. Indeed, the unusually high minimum for the decorticated seeds agrees well with the data secured in earlier determinations with the coat intact, when the coat  $\text{CO}_2$  production and the reversal of the correlation between hypocotyl and cotyledons are taken into consideration.

The lowering of the oxygen minimum by increase of temperature is at least partially accounted for by the increase in anaerobic respiration as the temperature rises. On the other hand, if a constant free oxygen supply below the optimum is maintained during a rise in temperature, the growth capacity of the organism is increased as the temperature rises. It is known, also, that merely cutting off the supply of oxygen will increase the anaerobic respiration in many organisms. With the oxygen reduced to a minimum, and at the high temperature employed, the conditions in these experiments are favorable to a considerable increase in the anaerobic form of respiration. As the anaerobic respiration increases, there is less need of the aerobic to release the energy sufficient to initiate growth. The question then naturally arises whether the lowering of the oxygen minimum by increasing the temperature really indicates any difference in the amount of respiration occurring. It is possible that the total energy release necessary for the germination of the seed is practically the same at any given temperature, the variation being in the proportion of release due to aerobic

and anaerobic respiration. This question can be answered only by further investigation.

A detailed study of the permeability of the seed coats of *Xanthium* to oxygen and other gases and reagents will be carried on, with the purpose of determining whether BECQUEREL's conclusions regarding the rôle of dry seed coats have general applicability.

The necessity for more exact quantitative studies of the factors which cause delayed germination is emphasized by this work. The factors which cause specific behavior of the seed are sometimes very minute and may escape detection entirely unless the methods employed in investigation are adapted to that end. The most refined methods of quantitative study are best suited to this purpose, and for a further advance with the problems of delayed germination it will be necessary to adopt the most exact and rigorous methods of analysis.

### Summary

1. The naked embryos of the dimorphic seeds of *Xanthium* exhibit a marked difference in their demand for oxygen for germination.

2. The oxygen minimum for the germination of decorticated *Xanthium* seeds at 21° C. is approximately 12 mm. for the upper seeds, and about 9.5 mm. for the lowers.

3. Increasing the temperature decreases the minima, a rise of 10° from 21° lowering the necessary minimum of oxygen from 12 mm. to approximately 7 mm. for the uppers, and from 9.5 mm. to approximately 3 mm. for the lowers.

4. Variation of the total atmospheric pressure probably does not influence the oxygen minimum for germination. The experiments indicate that equal partial pressures of oxygen produce approximately the same effect on the seeds, regardless of the total pressure of which it forms a part.

5. There is very little after-ripening, or at least the after-ripening is not visible in an altered germination behavior at atmospheric pressure and ordinary temperatures. There is evidence either of a decrease in the oxygen need, or an increase in the permeability of the coats to oxygen, or both, as ripening progresses.



6. A very slow progressive deterioration of the seeds takes place, which after a few years causes entire loss of power to germinate.

7. The general conclusion that the organs of the seeds of higher plants can grow in entire absence of free oxygen is not supported by the results obtained with *Xanthium* seeds. They cannot grow without comparatively large amounts of free oxygen.

8. The oxygen pressures required for germination of *Xanthium* seeds are very much higher than those reported by LEHMANN for the epicotyls of such plants as *Helianthus perennis*, *Zinnia elegans*, and *Glyceria fluitans*.

9. Since the coats cause delay by excluding oxygen, we might expect to find the oxygen demand for growth high. *Xanthium* seeds stand at the opposite end of the series from the seeds of certain aquatic plants, as water plantain and rice, in demands for oxygen for germination.

10. The high oxygen demand, and the difference in this demand in the two seeds, act with the coats to secure delay, and a difference in delay, in the two seeds. But if the coat has been removed, the demand for oxygen by the embryo is too low to be significant in securing delay in germination.

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